AmphetaminePromotes Recovery From Sensory–Motor Integration Deficit After Thrombotic Infarction of the Primary Somatosensory Rat Cortex

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The present studies were undertaken to examine 1) whether d-amphetamine sulfate administered to rats well after thrombotic infarction of the vibrissal cortical barrel-field within the primary somatosensory cortex affected the rate and completeness of behavioral recovery and 2) whether a dose–response relation exists between d-amphetamine sulfate dose and recovery of function. In a learning task requiring sensory–motor integration, 41 rats were trained to perform a motor response in a T-maze consequent to the detection of a vibrissal deflection cue. Once training was complete, unilateral (n=29) or sham (n=12) infarction was produced by a noninvasive photochemical technique. After infarction, T-maze performance was assessed repeatedly in rats receiving 2 (n=10) or 4 (n=10) mg/kg d-amphetamine sulfate or saline (n=9) 24 hours prior to testing on days 4, 6, 9, and 11. The sham-operated control rats received d-amphetamine sulfate (n=7) or no injections (n=5). All three infarcted groups displayed a reliable and sustained behavioral deficit in performance that was not present in the sham-operated control animals. Although the performance of each infarcted group improved over the testing sessions after the first injection, the amphetamine-treated groups improved at a faster rate than the saline-injected group. The results further demonstrated a dose–response effect, with the 4 mg/kg amphetamine group recovering to within preinfarction levels 6–8 days earlier than the 2 mg/kg amphetamine and saline-injected groups. Moreover, both amphetamine-treated groups recovered more completely than the saline-injected group. Quantification of the chronic infarct area revealed no differences among the amphetamine-treated and saline-injected groups. These data provide further evidence of the facilitatory effect of d-amphetamine sulfate on recovery from brain injury and extend this effect to the enhancement of recovery subsequent to thrombotic infarction of the primary somatosensory cortex. (Stroke 1991;22:648–654)

Since the turn of this century, the remarkable capacity for the brain to recover from cerebral injury, such as stroke, has spurred interest in the use of animal models to study mechanisms of functional recovery and apply them in the clinical setting.1 In recent years, data from animal studies and preliminary clinical findings indicate that some pharmacologic manipulations that alter neurotransmitter (e.g., acetylcholine, γ-amino butyric acid, serotonin, dopamine, and norepinephrine [NE]) function may markedly promote recovery of function following brain injury.2,3 One promising agent, the catecholamine agonist amphetamine, has been consistently shown during the past 20 years to reinstate locomotor, righting, and other postural reflexes and enhance recovery from learning and memory deficits induced by electrolytic brain lesions.2,3 In particular, one recent study found that a single dose of amphetamine given to cats 10 days after unilateral motor cortex ablation accelerated the rate of recovery of beam-walking ability compared with saline.4 When amphetamine was injected at 4-day intervals from the 10th to
the 22nd day following cerebral injury, an even greater increase in the rate of recovery was produced than after a single injection. More recently, a clinical study of humans presenting with symptoms of hemiparesis consequent to nonhemorrhagic stroke reported that a single dose of amphetamine improved motor performance during physical therapy. These findings suggest that the motor deficit and spontaneous recovery from unilateral motor cortex ablation may respond to pharmacologic treatment well after the onset of the cerebral insult.

We have examined behavioral deficit and recovery following thrombotic cerebral infarction, using a closed-skull preparation. This animal model uses a photochemical method to induce a cerebral infarct that produces morphologic and hemodynamic consequences in a cascade of stroke-like ischemic events. The temporal features of these events have been well documented. The capacity for a pharmacologic agent to facilitate recovery of function after the acute phase of injury may represent an important development for therapeutic intervention during the postsroke period. Therefore, the present study was undertaken to determine whether amphetamine given on four occasions (4, 6, 9, and 11 days after unilateral infarction of the primary somatosensory cortex) altered the rate and completeness of recovery of performance of a learning task requiring sensory–motor integration of vibrissal stimulation. By varying the dosage of amphetamine administered, we also sought to examine whether any relation existed between drug dosage and behavioral recovery.

**Materials and Methods**

We used 41 male Wistar rats weighing 250–400 g. The animals were housed in individual cages and maintained on a 12 hours light/12 hours dark cycle with water available ad libitum. The rats were randomly assigned, with 29 receiving unilateral infarction of the left primary somatosensory cortex and 12 receiving a sham operation. Prior to testing, the animals were reduced to 80% of body weight and maintained at this level throughout the study. In addition, food deprivation preceded testing by 24 hours.

The rats were tested in a T-maze consisting of a start chamber and a T-shaped goal chamber. The T-maze apparatus and vibrissal stimulator probe have been described. A trial began with the animal in the start box and ended with the arrival of the rat at either end of the T-maze. While in the start box, an animal received either right vibrissal stimulation or no vibrissal stimulation. Vibrissal stimulation consisted of a single caudal-to-rostral stroke with the tip of the probe delivered toward but not contacting the right side of the snout when the rat's head was centered in the start box facing the guillotine door. A trial was repeated if the probe contacted any part of the animal's body other than its vibrissae. The rats were trained to turn right for food reinforcement (45 mg, Noyes Precision Pellets, Lancaster, N.H.) only if they had previously received vibrissal stimulation in the start box. If they had received no vibrissal stimulation prior to the guillotine door opening, then the animals were required to turn left in the T-maze for food reinforcement. These left-turn trials served to control for the detection of vibrissal stimulation. Since red light is not visible to rats, all testing was performed in the dark, with only indirect red light illumination (Kodak safelight filter 1A, Rochester, N.Y.) available for the experimenter. Therefore, by eliminating any extraneous sensory cues, the presence or absence of right vibrissal stimulation served as the only cue the animals could use to determine the correct direction to turn and subsequently receive food reinforcement.

The rats were tested every other day and at each session received 100 randomly ordered trials divided equally between 50 left and 50 right turns. Correct responses (i.e., vibrissal stimulation–right turn or absence of vibrissal stimulation–left turn) were reinforced immediately with the delivery of a food pellet. The behavioral criterion was achieved and training was concluded when the rats responded correctly on >80% of the right- and >80% of the left-turn trials for three consecutive testing sessions. These last three sessions served as the animal’s baseline performance against which postsurgical testing was compared.

After unilateral or sham infarction, the rats were repeatedly tested every other day for 35 days, with 100 trials per session. Postsurgical testing began on the third day to establish whether a behavioral deficit was present. Infarcted animals were then divided randomly into three treatment groups and received 2 mg/kg i.p. d-amphetamine sulfate (d-AMP) (Sigma Chemical Co., St. Louis, Mo.) (n=10), 4 mg/kg i.p. d-AMP (n=10), or intraperitoneal saline (n=9). Sham-operated rats received either 2 or 4 mg/kg i.p. d-AMP (n=7) or no injections (n=5). The control group comprises all 12 sham-operated rats. All injections were given approximately 24 hours prior to testing on each of postsurgical days 4, 6, 9, and 11. Experimenters were blinded to the surgical and pharmacologic treatment each animal received.

Photochemical cerebral infarction was induced only after the behavioral criterion was reached. A detailed account of the apparatus and procedure for inducing photochemical infarction has been described. Briefly, in the infarcted groups, photochemical infarction was induced in halothane-anesthetized rats by infusing 20 mg/kg i.v. rose bengal dye through a tail vein catheter for 2 minutes and then irradiating the cranium for 7 minutes at 7.2 mm anterior to the interaural line and 5.0 mm lateral to the midline directly over the left cortical barrel-field of the primary somatosensory cortex. Sham-operated rats underwent the same procedure as the infarcted animals, except isotonic saline instead of rose bengal dye was infused and consequently no photochemical reaction took place.

At the end of the study, 40–45 days after surgery, the rats were deeply anesthetized with halothane and
perfusion-fixed with formaldehyde, glacial acetic acid, and methanol. The brains were left in situ overnight before removal from the cranial vault. The brains were then stored in the perfusion solution until they were blocked, dehydrated, and infiltrated with and embedded in paraffin. Coronal sections 10 μm thick were cut and stained using hematoxylin and eosin. The lesion's epicenter and the area of maximal cortical necrosis were first determined by light microscopy of multiple stained sections. The tracing of each histologic section was subsequently redrawn onto a digitizing tablet (Summagraphics Corp., Seymour, Conn.) interfaced with a MicroVax minicomputer, which computed the areas.

The data were assessed to determine whether d-AMP administration affected T-maze task performance relative to baseline performance. The mean percentage of correct responses for the three baseline sessions was subtracted from the percentage of correct responses for each postsurgical session to obtain an index of performance relative to baseline. Behavioral deficit was established as the difference between the mean baseline percentage and the postsurgical day 3 percentage of correct responses. Behavioral recovery was assessed using the BMDP computer statistical package with repeated-measures analysis of variance. Analyses were performed to assess differences between groups (2 mg/kg d-AMP, 4 mg/kg d-AMP, saline, and sham-operated controls) in the rate of recovery over postsurgical days 3 to 35. An additional measure of behavioral recovery was obtained by comparing the postsurgical day when performance recovered to within 10% of baseline to assess the temporal influence of d-AMP treatment.

Results

A consistent pattern of cortical necrosis was demonstrated in both d-AMP- and saline-treated rats. Figure 1 displays sections through the infarct epicenter (7.2 mm anterior to the interaural line) from representative d-AMP- and saline-treated rats. Infarcts were well-demarcated and appeared cystic. In addition to a glial scar, the infarct contained macrophages, astrocytes, and blood vessels. The chronic infarcts commonly extended 9.2 mm anterior and 4.7 mm posterior of the interaural line; the medial border was 4.0 mm lateral to the midline, and the lateral border was 5.0 mm dorsal to the interaural line. Quantitative analysis of infarct areas demonstrated no significant differences between saline-treated (2.5±0.2 mm²) and d-AMP-treated (2 mg/kg 2.0±0.2 mm², 4 mg/kg 2.2±0.2 mm²) rats.

Analysis of baseline performance showed no significant differences between the infarcted and control groups. The mean±SEM percentage of correct responses (baseline performance) for all groups was 89.0±0.6%.

Figure 2 depicts the change in mean percentage of correct responses per session from postsurgical day 3 to 35 for all groups. The analysis of behavioral deficit comparing the infarcted and sham-operated groups' performance during baseline with postsurgical day 3 performance revealed a significant interaction between groups over testing days (F(1,37)=10.8, p<0.001). No difference among the sham-operated subgroups was found (data not shown), although a small but significant behavioral deficit was observed (F(1,9)=13.6, p<0.01). The infarcted groups combined also displayed a significant behavioral deficit (F(1,26)=277.3, p<0.001), obtaining only 61.6% of the trials correct, a behavioral performance at or just above the level of chance. An analysis of only the postsurgical day 3 performance of the infarcted groups combined revealed a greater behavioral deficit (27.0±1.6%) than the control group (9.9±2.9%) (F(1,37)=33.6, p<0.001; Figure 2).

Analysis of the effect of d-AMP on the control group's performance from day 3 to 35 revealed no significant subgroup differences, and therefore data for the 2 mg/kg d-AMP, 4 mg/kg d-AMP, and no injection sham-operated rats were combined. Rates of recovery of performance over postsurgical days 3 to 35 differed in the control and infarcted groups (F(3,37)=21.2, p<0.001; Figure 2). The behavioral deficit in the control group was short-lived, and by day 5 (the second testing session after sham surgery), per-
performance did not differ significantly from baseline. In contrast, the behavioral deficit of the infarcted groups was more prolonged, lasting 1–2 weeks, before performance gradually recovered toward baseline (Figure 2). Repeated-measures analysis of variance of the three infarcted groups' performance from day 3 to 35 yielded a significant interaction between treatment and postinfarction day ($F_{(2,30)}=3.5, p<0.05$). When the two d-AMP-treated groups were compared from day 3 to 35, no differences emerged, although a significant increasing linear trend over postinfarction days was found ($F_{(4,64)}=214.1, p<0.001$), indicating that the two d-AMP-treated groups recovered at the same rate. However, when the saline-treated group was compared with either d-AMP-treated group, significant differences in the recovery of performance over postinfarction days was observed ($F_{(1,17)}=5.4, p<0.05$ for 2 mg/kg d-AMP; $F_{(1,17)}=4.5, p<0.05$ for 4 mg/kg d-AMP). Therefore, d-AMP at either dose resulted in greater acceleration of the rate at which performance improved after unilateral cerebral infarction compared with saline.

Figure 3 displays the mean±SEM post-surgical day when performance recovered to within 10% of baseline for all groups. The analysis of these data revealed a significant difference among groups ($F_{(2,20)}=33.3, p<0.001$). The control group recovered by about the second postsurgical testing session (5.1±0.6 days). When the infarcted groups were compared, a significant treatment effect ($F_{(2,20)}=3.7, p<0.05$) was observed. As seen in Figure 3, the 4 mg/kg d-AMP group recovered sooner than the saline-treated group ($F_{(2,20)}=6.2, p<0.05$) or the 2 mg/kg d-AMP group ($F_{(2,20)}=6.2, p<0.05$). No significant difference was found between the saline-treated and 2 mg/kg d-AMP groups. Therefore, the high-dose d-AMP group recovered to within 10% of baseline significantly earlier (19.4±1.4 days) than either the low-dose d-AMP (25.6±2.0 days) or the saline-treated (27.5±3.0 days) group.

To determine whether d-AMP affected the overall level of recovery by the end of the testing period, another analysis assessed differences between groups from day 33 to 35. Both d-AMP-treated groups displayed a more complete behavioral recovery than the saline-treated group ($F_{(1,17)}=4.57, p<0.05$ for 2 mg/kg d-AMP and $F_{(1,17)}=4.41, p<0.05$ for 4 mg/kg d-AMP). Mean±SEM changes on days 33–35 from baseline of 0.4±1.9%, -0.2±1.4%, and -8.1±3.7% were observed for the 4 mg/kg d-AMP, 2 mg/kg d-AMP, and saline-treated groups, respectively (Figure 2).

**Discussion**

Using combined pharmacological and behavioral strategies in the present study, we show that d-AMP, when administered in multiple injections well after the cerebrovascular insult, facilitates behavioral recovery from a sensory–motor integration deficit sub-
sequent to thrombotic infarction of the primary somatosensory cortex in rats. Unilateral infarction of the cortical barrel-field contralateral to vibrissal stimulation produced a large decrease in the percentage of correct behavioral responses, with the accuracy of the infarcted animals dropping to the level of chance at the first postinfarction session. The behavioral deficit exhibited by these rats was not due to any motor deficit since they still performed the task, albeit inaccurately. Moreover, the behavioral deficit probably did not result from a loss of the animals' ability to sense vibrissal deflection since, as indicated by the reflexive inhibition of an ongoing motor behavior. Instead, the inability to produce the correct motor response was more akin to a vibrissal agnosia; the rats simply were unable to integrate the vibrissal deflection with the correct motor response. The saline-treated infarcted animals in this study displayed a gradual improvement in performance from postinfarction days 3 through 35, recovering to within 10% of baseline between 25 and 35 days following cortical injury. It should be noted that in previous studies, as well as in the current study, although there was a significant improvement in performance, the infarcted animals did not recover completely. Thus, some performance deficit remained long after the cerebral ischemic event was induced.

The major focus of this study was whether d-AMP influenced the recovery of function after cerebral infarction of the primary somatosensory cortex. At both 2 and 4 mg/kg, d-AMP augmented the rate of behavioral recovery relative to saline. Moreover, the high-dose d-AMP group recovered to within 10% of baseline earlier, by the 19th day following infarction, than the low-dose d-AMP and saline-treated groups, which recovered to within 10% of baseline by about days 26 and 28, respectively. Recovery in the d-AMP-treated animals not only approached, but matched, baseline, indicating more complete recovery than the saline-treated rats, in which performance remained about 8% below baseline. Therefore, behavioral recovery occurred more quickly and more completely in the infarcted rats receiving d-AMP than in those receiving saline.

The ability of the high dose of d-AMP to accelerate behavioral recovery is consistent with previous findings of d-AMP-accelerated behavioral recovery from motor cortex ablations in both rats and cats performing motor tasks requiring more basic and complex initiation and modulation of movement. More recent studies have found that single or multiple injections of 2 mg/kg d-AMP given after unilateral motor cortex ablation enhanced the rate of recovery of beam-walking compared with saline, with multiple injections yielding a more rapid recovery than a single dose. A recent dose–response study employing this same behavioral task reported that d-AMP affects recovery over a narrow range of doses.

The d-AMP dose with optimal effect on beam-walking recovery was 3.5 mg/kg; lower doses had less or no effect on recovery, and higher doses elicited behavioral stereotypes that were suggested to interfere with locomotion, and consequently high-dose d-AMP was less effective in modifying recovery. These dose–response findings corroborate ours; we found a greater facilitation of behavioral recovery with 4 mg/kg d-AMP than with 2 mg/kg d-AMP.

The relatively high doses of d-AMP required to promote recovery can affect NE, dopamine, and serotonin neurotransmission, thus implicating these substances in postinjury recovery. Adrenergic agonists activate locus ceruleus noradrenergic neurons, resulting in sustained stimulation of cortical NE release and neuronal excitability. The beneficial effects of d-AMP on rat motor recovery are blocked by haloperidol, a D2 dopamine receptor–blocking agent, which is often given to counter agitation in stroke patients. Thus, dopamine may play a role in d-AMP-facilitated recovery. Consistent with this notion are data showing that the D2 agonist bromocriptine may enhance functional recovery following brain injury. However, it is possible that the beneficial effect of dopamine is the result of its being taken up by presynaptic NE terminals and metabolized to NE. The dopamine agonists apomorphine and methylphenidate have weak or no effects on motor recovery. In contrast, the α1-adrenergic antagonists idazoxan and yohimbine, but not the α2-adrenergic antagonist prazosin nor the β-adrenergic antagonist propranolol, improved motor function following unilateral sensory-motor cortical damage. Indeed, a single dose of NE enhances motor recovery but has only a transient facilitatory effect if the adrenergic nucleus, the locus ceruleus, has been previously lesioned. Taken together, these findings suggest that amphetamine-accelerated recovery may be mediated by noradrenergic rather than dopaminergic neurotransmission.

Since d-AMP-treated rats in beam-walking studies appear to recover by the first testing session, it is improbable that the behavioral recovery is due to structural changes proximal to the site of injury. Alternatively, the behavioral deficit observed in beam-walking may, as has been previously suggested, be the result of a generalized depression of subcortical structures related to the motor pathways that were required to perform the motor response. That is, the initial behavioral deficit may arise from the lesioned area and functional depression of neural structures remote from but connected to the site of injury. Studies in our laboratory using the photochemical model of cerebral infarction have revealed a temporal pattern of widespread pathophysiological change. Following unilateral cortical infarction, consistent with the notion of remote functional depression, an acute decline of hemodynamic and metabolic function occurs within
the same hemisphere both proximal to and remote from the site of primary injury; this decline begins to resolve gradually within the first or second postinfarction week. Most spontaneous improvement in performance on a vibrissal-discrimination task occurs after the first 10–12 postinfarction days.10 Of note in the current study was the performance of infarcted rats that received 4 mg/kg d-AMP on postinfarction days 4, 6, 9, and 11. When such rats emerge from this early postinfarction period, they appear to maintain a more steady and accelerated rate of recovery than other infarcted rats (Figure 2). Compared with the beam-walking studies, the deficit observed during the T-maze task is more prolonged and recovers more slowly, even after the administration of d-AMP, suggesting that in addition to resolution of remote functional depression, other mechanisms of recovery may be operating. Indeed, in awake rats undergoing a metabolic study of unilateral vibrissal stimulation 30 days following unilateral infarction of the primary somatosensory cortex, increased metabolic activity was observed to spread anterior and lateral to the site of injury in regions of related somatosensory cortical function.16 This finding suggests that some underlying structural reorganization occurs during more chronic stages following stroke.

Recently it has been demonstrated that acute d-AMP administration in sham-operated control and cortical barrel-field–infarcted rats allows normally depressed secondary or alternate brain circuits to respond to unilateral vibrissal stimulation.9 Consequently, the d-AMP–mediated release of neuromodulators, such as NE, may provide a condition of increased cortical excitability that permits circuit modification in normal and postinjury states. The nonimmediate, more delayed effect of d-AMP on behavioral recovery in the present study may be a product of the treatment-induced resolution of remote metabolic abnormalities. This in turn may provide access to secondary or alternate circuits that may facilitate recovery.36 It should be noted that in the beam-walking studies d-AMP did not facilitate recovery alone; recovery occurred only with the combination of d-AMP and motor experience.52 All rats in the present study could freely gain vibrissal experience in their home cages, in addition to the rigorous learning experience obtained during each testing session. Thus, the role of vibrissal experience should not be eliminated as a potential factor facilitating recovery, perhaps having the most impact once access to secondary or alternate circuits is acquired. Further delineation of the temporal sequence of underlying mechanisms operating in this animal model of stroke may have implications for issues of clinical concern, such as establishing optimal drug selectivity, dosage, and timing parameters for the initiation of pharmacotherapy.

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References


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